DEVELOPMENT OF LABORATORY MODEL ECOSYSTEMS AS EARLY WARNING ELEMENTS OF ENVIRONMENTAL POLLUTION

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December 1974

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER [redacted] Paper No. 1	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) DEVELOPMENT OF LABORATORY MODEL ECOSYSTEMS AS EARLY WARNING ELEMENTS OF ENVIRONMENTAL POLLUTION		5. TYPE OF REPORT & PERIOD COVERED Final Report
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Robert L. Metcalf, PhD		8. CONTRACT OR GRANT NUMBER(s) In part under Contract F33615-73-C-4059*
9. PERFORMING ORGANIZATION NAME AND ADDRESS Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F; 6302; 630201; 63020113
11. CONTROLLING OFFICE NAME AND ADDRESS Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433		12. REPORT DATE December 1974
		13. NUMBER OF PAGES 12
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

*Conference was arranged by the Toxic Hazards Research Unit of the University of California, Irvine

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Subject Field: 0620; 0615; 0606

Inhalation toxicology
Environmental toxicology
Environmental Carciogenesis
Pathology
Cellular toxicology

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Major technical areas discussed included ecosystem modeling, water pollution, water reuse, toxic hazards evaluation of fire extinguishants, environmental carcinogenesis, and cellular toxicology.

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AMRL-TR-74-125

PAPER NO. 1

DEVELOPMENT OF LABORATORY MODEL ECOSYSTEMS AS EARLY WARNING ELEMENTS OF ENVIRONMENTAL POLLUTION

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INTRODUCTION

Problems of environmental pollution with toxic substances, singly or in combination, have greatly intensified during the past three decades. In the 1940's we were told that the "solution to pollution was dilution" and we were confident that the capacity of our environment for absorption, dilution, and detoxication was infinite. Today we are deeply concerned about the parts per trillion levels of pollution of the Great Lakes with DDT, dieldrin, PCB's, Hg etc. and their 10^5 to 10^7 biomagnification in fish and consequent entry into terrestrial food chains and into the human diet (E. P. A., 1972). Trace contamination of agricultural soil at 0.1 - 1 ppm with dieldrin, the epoxidation product of the insecticide aldrin, has been picked up in cotton and soybeans, and introduced by plant oil sludges used as feed supplements into the diets of as many as 25 million chickens which have been destroyed because of the illegal residues of potent carcinogen that they contain (Randal, 1974). Traces of lead from tetraethyl lead in gasoline and cadmium from rubber chemicals in automobile tires have polluted our cities and the verges of our highways until our streets are literally paved with these metals and our house dust is unsafe to breathe (Ewing and Pearson, 1974).

Most of the source of our concern about environmental pollution by trace substances relates to the inventiveness of the chemical industry and to its exponential growth. The first edition of the Merck Index (1889), "containing a summary of whatever chemical products are today adjudged as being useful in either medicine or technology," listed about 828 chemicals, the eighth edition (1968) more than 10,000. The "Toxic Substances List" NIOSH, 1973) catalogues 25,043 potentially hazardous chemicals. Quantitatively, organic chemical production in the United States has risen from 10 billion pounds in 1943 to 140 billion pounds in 1972. The rate of growth is exponential, increasing at an average of about 9% per year (doubling time 8 years) and the 1972 production was equivalent to 676 pounds per capita.

These chemicals have almost endless uses as intermediates in synthesis, fuel and fuel additives, plastics, elastomers, plasticizers, brighteners, heat-transfer agents, flame retardants, rubber chemicals, paints, lacquers, solvents, fibers, detergents, insecticides, fungicides, herbicides, bactericides, coccidiostats, growth promoting agents, and pharmaceuticals. They enter the environment accidentally from industrial effluents, household applications, or as by-products of transportation; or purposefully from application of pesticides, protective coatings, and preservatives. It is estimated that from 500 to 700 new chemicals are produced each year on a scale large enough so that significant traces of them enter directly into the environment (Lee, 1964). As environmental micropollutants, these chemicals may be harmless, toxic, carcinogenic, mutagenic, or teratogenic. The effects may require many years to determine in the human population, e.g. the recent indictment of vinyl chloride (U. S. production 5.35×10^9 lb. in 1973) as a human carcinogen (CDC, 1974), the discovery of phthalate ester plasticizers (U. S. production 1×10^9 lb.) as teratogens (Singh et al., 1973), the implication of PCB residues in Lake Michigan fish as the cause of sterility of domestic mink (Ringer et al., 1972), and of atmospheric manganese emissions from mining, iron production, and battery production (U. S. manganese consumption 1.55×10^9 lb. in 1968) in producing manganism, a neurological disorder closely resembling parkinsonism (NAS, 1973). The most alarming implication of trace element pollution is a recent World Health Organization estimate that 85% of all human cancers have an environmental cause (C & E News, 1974).

These astronomical figures together with exponential growth of the U. S. and world chemical industries makes it easy to appreciate the necessity for a "Toxic Substances Act" such as that now under consideration by Congress. The proposed Act will require the manufacturer or user to provide proof of the environmental safety of the various "toxic substances." The proof will require the employment of various "early warning" techniques and it appears that some sort of model ecosystem which can demonstrate the degree of biodegradation and possible biomagnification and food chain transfer of "toxic substances" will become an important component of such "early warning" evaluations.

DEVELOPMENT OF LABORATORY MODEL ECOSYSTEM

Our laboratory became interested in 1966 in the development of a simple laboratory model ecosystem under a grant from the Herman Frasch Foundation, American Chemical Society. Our idea was to use radiotracer methodology to follow the movement and degradation of pesticides through a series of food chain transformations and to develop information about:

1) toxic effects of pesticide and its degradation products in organisms from at least 5 phyla.

2) degradative pathways of pesticide in the environment.

3) bioaccumulation of pesticide and its degradation products.

4) biodegradability of pesticide and its degradation products.

It was our purpose to obtain all of this information in a simple, inexpensive laboratory system which could be used in environmental toxicology in a manner analogous to the use of the rat in industrial toxicology.

Initially we chose to model the application of the pesticide to a farm, orchard, or forest, and to follow by the radiotracer technique the movement and fate of the compound into a lake. Real world analogues could range from application of a pesticide to a cornfield and its transfer into a farm pond or the application of DDT to elm trees in the Chicago area for the control of the bark beetle vectors of Dutch elm disease and the consequent dispersal into Lake Michigan. In the latter case, it is well known that DDT in the lake at 0.000002 ppm has become biomagnified into fish up to 10 - 20 ppm or 10^7-fold (EPA, 1972; Harrison, 1970).

The methodology of this system has been described in detail (Metcalf et al., 1971, 1974a, 1974b). Very briefly it is housed in a 10 x 10 x 20" glass aquarium containing a sloping shelf of washed white sand. The lower portion is covered with 7 liters of standard reference water which forms a "lake" in which are grown plankton, alga (Oedogonium carciacum), Daphnia magna, mosquito larva (Culex pipiens), snail (Physa sp), and fish Gambusia affinis) which provide the test organisms and food chain components of the system. On the flattened terrestrial portion are grown Sorghu · vulgare plants which provide the "farm" portion of the ecosystem.

The aerated aquarium is partially covered with a plexiglas cover to retard evaporation and is placed in an environmental plant growth chamber at 80 F with a 12-hour simulated daylight of 5000 foot candles.

Several different protocols of operationof this model ecosystem have been developed: (1) the typical pesticide evaluation involves quantitative application of 1 to 5 mg (0.2 to 1 lb./A. equivalent) of the radiolabeled pesticide (^{14}C, ^{3}H, ^{32}P, ^{35}S) from acetone solution to the leaves of the Sorghum. The treated plants are then eaten by the salt marsh caterpillar Estigmene acrea and this serves as the dispersing agent for the labeled micropollutant. (2) The radiolabeled micropollutant is injected by micro-pipette as an acetone

solution into the sand of the terrestrial portion to simulate a pre-emergent herbicide or is applied to Sorghum seeds to simulate seed treatment with insecticide or fungicide. (3) The radiolabeled micropollutant is added directly to the water portion to simulate an aquatic effluent pollutant. (4) The micropollutant is incorporated directly into standard soil types which is added to the sand to simulate such problems as lead pollution along the highway, addition of sewage sludge to crop land, or soil herbicide or insecticide adsorption and erosion. In place of radiolabeling, atomic absorption spectrometry or neutron activation analysis can be used for the ultimate analysis.

Following any of these methods of application, the model ecosystem units are left for 33 days and at the end of the experiment the organisms are sampled and counted for total radioactivity, extracted with organic solvents and the extracts separated into chemical constituents by thin layer chromatography (TLC) and radioautography, followed by liquid scintillation counting to quantitate the results. From this workup we obtain (a) the ecological magnification (E.M.) or ppm of parent compound (or a particular degradation product) in organism/ppm in water; (b) the biodegradability index (B.I.) or ppm of polar radioactivity in organism/ppm of non-polar radioactivity (from TLC plates), (c) the degradative pathways from determination of the chemical nature of degradative products by cochromatography with known model compounds, mass spectrometry, etc, and (d) the unextractable or totally metabolized radioactivity.

Applications of Terrestrial Aquatic Model Ecosystem

We have employed the model ecosystem as described above in several major ways.

1. Evaluation of pesticides intended for agricultural use in Illinois.

In cooperation with the Illinois Natural History Survey we are routinely screening all pesticides intended for use in Illinois and to date have completed evaluation on about 60 pesticides. Illinois corn and soybean culture is one of the heaviest users of pesticides in the world and the magnitude of the task is indicated by the introduction under experimental permit of 10 new compounds in 1974. Essentially nothing is known about their environmental toxicology and fate. Unfortunately this is also the situation with many of the older well established pesticides. Model ecosystem studies of a number of organochlorine insecticides have been reported (Metcalf et al., 1973). As we increase the number and range of pesticides evaluated we will be able to project rather accurately the environmental effects to be expected from compounds with E. M. values and B. I. values in particular ranges.

2. Evaluation of insecticides intended for vector control by WHO.

The problems of vector resistance to the older insecticides such as DDT and lindane, and new and important vector-control programs, e. g. the $100 million onchoceriasis control program in Mali and Upper Volta of Africa (WHO, 1973), have created an urgent demand for new types of insecticides. There is an important need to demonstrate their safety and biodegradability, especially for compounds applied directly to water for control of the blackfly larva Simulium damnosum, vector of Onchocerca volvulus.

WHO has requested our International Collaborative Center for Insecticides at the University of Illinois to undertake model ecosystem evaluation of all new pesticides intended for field experimentation in vector control. To date we have evaluated approximately 20 insecticides, including methoxychlor and other DDT analogues, organophosphorus and carbamate insecticides, and insect growth regulators. The evaluation of chlorpyrifos (Dursban®) and its methyl analogue is illustrative. Chlorpyrifos (OMS-971) is one of the most effective larvicides found in the WHO "Programme for the Evaluation and Testing of New Insecticides" (WHO/VBC/71, 1971). However, it is undesirably toxic to humans (rat oral LD_{50} 135 mg/kg) and its O,O-dimethyl analogue, methyl chlorpyrifos (OMS-1155), which is nearly as effective as a larvicide and much safer (rat oral LD_{50} 1000 mg/kg), was also considered for use (Quellenec, 1972). A decisive factor was the relative persistence and biodegradability of the two compounds. With the excellent cooperation of the Dow Chemical Company, ^{14}C-radiolabeled chlorpyrifos and methyl chlorpyrifos were compared in model ecosystem evaluations (Metcalf, 1974). The results showed that methyl chlorpyrifos, E.M. 95, B. I. 3.95, was much more biodegradable and less biomagnified than chlorpyrifos, E.M. 314, B. I. 1.02.

3. Development of Principles of Biodegradability and New Biodegradable Insecticides. Our laboratory, sponsored by a grant from the Rockefeller Foundation for the "Development of Novel, Non-Persistent Insecticidal Compounds" has been studying the effects of incorporating degradophores or groups serving as substrates for the microsomal oxidase enzymes, into the basic structure of the DDT type molecule. Quantitative data on E.M. and B.I. from laboratory model ecosystem studies for more than 15 DDT analogues altered in both aromatic and aliphatic positions has been essential in demonstrating biodegradability and exploring degradative pathways (Metcalf et al., 1972).

4. Evaluation of the Micropollutant Properties of Toxic Substances.

As new alarms are raised about the distribution and fate of newly discovered micropollutants, the model ecosystem approach has repeatedly demonstrated its value in producing specific data about bioconcentration, food chain transfer, degradation pathways, and toxicity levels to various organisms. During the past 5 years we have conducted model ecosystem studies of such new pollution problems as mirex, hexachlorobenzene (Metcalf et al., 1973a) TCDD, dioctyl phthalate (DOP) plasticizer (Metcalf et al., 1973b), and the polychlorinated biphenyls (PCB's) (Metcalf et al., 1974). The PCB studies were conducted with pure ^{14}C-radiolabeled tri-, tetra-, and pentachlorophenyls and demonstrated the pronounced decrease in degradative pathways which resulted from an increasing number of chlorine atoms. Thus the biomass recovery with trichlorobiphenyl was 0.45%, with tetrachlorobiphenyl was 8.7%, and with pentachlorobiphenyl was 57.2%. No evidence could be detected of the environmental conversion of DDE to PCB type compounds.

PROLIFERATION OF THE MODEL ECOSYSTEM CONCEPT

The wealth of information readily available from the radiotracer investigations of micropollutants in small model ecosystems and the urgent demands for information about the environmental behavior and fate of chemical contaminants has resulted in extension of the model system concept far beyond our original proposal. Today we have investigations in progress with at least four modifications of the original system, dealing with pesticides, plasticizers, veterinary drugs, carcinogens, food additives, industrial chemicals, trace metals, and organic impurities.

Model Aquatic Ecosystem

The effluents from industrial manufacturing contain thousands of organic chemicals whose environmental fate requires investigation. These ultimately find their way through sanitary sewers, dumping, etc. into the aquatic environment. The model aquatic ecosystem was devised to measure bioaccumulation and food chain movement of such contaminants using as components the same organisms as used in the terrestrial-aquatic ecosystem. The system is totally enclosed in a 2-liter round-bottom 3-neck flask fitted with traps for organic vapors and CO_2. Thus it is possible to investigate relatively volatile compounds, including vinyl chloride, and to obtain quantitative data on the total breakdown and distribution of the contaminant. Using this system, we have studied the environmental fate of a series of simple benzene derivatives: aniline, anisole, benzoic acid, chlorbenzene, nitrobenzene, and phthalic anhydride, and the specialty chemicals hexachlorobenzene, pentachlorophenol, 2,6-diethylaniline and 3,5,6-trichloro-2-pyridinol

(Metcalf and Lu, 1973; Lu, 1974). Quantitative relationships were developed between the intrinsic molecular properties of the contaminants and the biological responses. Water solubility was related to ecological magnification with a correlation constant r = -0.92 and the octanol/H_2O partition constant pi to ecological magnification, r = 0.79. Considerable attention was given to the comparative degradation pathways in organisms of the several phyla. It was found that electronic properties (sigma constant) for the various benzene derivatives were related to the rate of degradation and loss of the compounds in the organisms, with a correlation constant r = 0.91 (Lu and Metcalf, 1975). This work provides a theoretical basis for relating basic molecular characteristics to environmental behavior.

Rice Paddy Model Ecosystem

Rice is the world's most important crop and is grown in peculiarly intimate aquatic-terrestrial environment. Rice culture is extraordinarily intensive and a very large variety of pesticides, herbicides, insecticides, and fungicides are used in rice culture. For example, in Taiwan in 1969 approximately 3 kg of pesticides per ha. were applied to the rice crop, representing 60% of the total pesticide consumption on the island. More than 200 pesticide chemicals are used and the problem is complicated by the multi-cropping system with 2 or 3 crops yearly, and by the importance of fish culture in rice paddies. Highly persistent chemicals such as BHC and pentachlorophenol have seriously affected rice culture in Japan (Goto, 1971). The rice paddy model ecosystem represents only a minor modification of the terrestrial aquatic system, and uses a higher water table with rice, Oryza sativa, grown on sand. A completely screened cover, with removable hatch permits using rice leafhoppers and lepidopterous pests as typical components of the rice-ecosystem. Goldfish, Carassius auratus, are used as representatives of the carp family. Problems currently under investigation include interactions between esterase inhibitors such as the insecticide carbaryl which inhibits the degradation of the rice herbicide propanil, so that injury to rice results when they are used together (Kaufman et al., 1970). Such problems of pesticide interaction are particularly amendable to study in model ecosystems, using combinations of labeling, i.e. with ^{3}H and ^{14}C.

Veterinary Drug Model Ecosystem

This research project (supported by the Food and Drug Administration Contract 74-127) has just begun and is planned to model the administration of veterinary drugs such as coccidiostats, growth hormones, antibiotics, and feed supplements to animals in food lots with consequent loss of the drugs and their metabolic products into the environment. A modification of the terrestrial aquatic model ecosystem in which the drug is administered to baby chicks or white mice caged above the terrestrial "farm" area is being used. Compounds under study initially include diethyl stilbestrol, selenium, and phenothiazine.

Terrestrial Model Ecosystem

This research project, just inaugurated (supported by the Environmental Protection Agency Grant R 803249), involves the application of radiolabeled pesticides to three typical agricultural environments: corn-soil, soybean-soil, and cotton-soil. The "ecosystem" consists of a 5 gallon wide mouthed jar in which 50 plants are grown in vermiculite or soil-vermiculite mixtures with synthetic water. The jars contain typical invertebrate populations of insects, earthworms, slugs, and isopods, and the food chain transfer is completed to the mouse (Microtus) as a general omnivore. Samples are taken at intervals of the soil, the plant, and the air to determine the total fate and balance of the radiolabeled compound under investigation.

CONCLUSIONS

To date we have studied the fate of approximately 100 organic compounds in the several types of model ecosystems described. This has given us a considerable background of data to relate the behavior of the compound in the model system to that in the real environment. We believe that the use of quantitative parameters such as ecological magnification (E.M.) and biodegradability index (B.I.) will enable us to relate any new or potential pollutant to well characterized compounds, and that from such information we can predict areas of probable environmental concern. The use of the model ecosystem technology provides a relatively simple way to measure the environmental toxicology and degradation pathways for new compounds in a variety of organisms. Model ecosystems are invaluable for screening new compounds for environmental persistence and degradation to demonstrate which of many possible derivatives or modifications are least hazardous to environmental quality. Model ecosystems provide the only practical way to study the complex interactions involved in the biological behavior of mixtures of environmental pollutants. Finally, model ecosystem studies are unusually graphic and visually appealing and have considerable teaching value for study of the problems of environmental micropollution.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge the financial support of the following agencies. Herman Frasch Foundation American Chemical Society; The Rockefeller Foundation; The World Health Organization; the Water Resources Center, University of Illinois and the U. S. Department of Interior, Contract B-050 Illinois; the U. S. National Science Foundation, RANN Program, Grant GI 39843X; the U. S. Department of Health, Education, and Welfare, Food & Drug Administration, Bureau of Veterinary Medicine, Contract FDA 72-116, and Contract 74-127; and the U. S. Environmental Protection Agency, NERL - Corvallis, Grant R 803249; the Illinois State Natural History Survey.

The following students and colleagues have participated in various phases of the work reviewed: Dr. J. N. Sanborn, Dr. I. P. Kapoor, Dr. A. S. Hirwe, Dr. Po-Yung Lu, Dr. An-horng Lee, Dr. Joel Coats, Dr. Larry Cole, Dan Brown, Carter Schuth, Nancy Plummer, Pat Sherman, Rick Furman, Alan Tegen, Maggie Yang, Caroline Change, Ernie Hsu, and Janet Williams.

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